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FILE 'REGISTRY' ENTERED AT 12:35:58 ON 01 MAR 2007

L1 1 S 145-50-6/RN  
L2 1 S 569-61-9/RN  
L3 2 S L1 OR L2

FILE 'CAOLD, CAPLUS, CASREACT, CROPU, DGENE, DPCI, ENCOMPPAT, EPFULL, FRANCEPAT, FRFULL, FSTA, GBFULL, IFIPAT, IMSPATENTS, INPADOC, JAPIO, KOREAPAT, LITALERT, NTIS, PAPERCHEM2, PATDD, PATDPA, PATDPAFULL, PATDPASPC, PCTFULL, PCTGEN, PIRA, PROUSDDR, PS, ...' ENTERED AT 12:36:34 ON 01 MAR 2007

L4 761 S L3  
L5 4 S L4 AND KIT? AND AMMONIA?

=> display bib hit l5 1-4

L5 ANSWER 1 OF 4 USPATFULL on STN  
AN 2006:95183 USPATFULL  
TI Enzymatic substrate, synthesis method and uses  
IN Armstrong, Lyle, Northumberland, UNITED KINGDOM  
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PA Bio Merieux, Marcy l'Etoile, FRANCE (non-U.S. corporation)  
PI US 7029886 B1 20060418  
WO 2001030794 20010503  
AI US 2000-111674 20001025 (10)  
WO 2000-FR2971 20001025  
20020726 PCT 371 date  
PRAI FR 1999-13756 19991028  
DT Utility  
FS GRANTED  
EXNAM Primary Examiner: Tate, Christopher R; Assistant Examiner: Garcia, Marcela M Cordero  
LREP Oliff & Berridge, PLC  
CLMN Number of Claims: 50  
ECL Exemplary Claim: 1  
DRWN No Drawings  
LN.CNT 1074  
CAS INDEXING IS AVAILABLE FOR THIS PATENT.  
AB The invention concerns novel enzymatic substrates of general formula (I) wherein: X represents a nitrogen atom or a carbon atom substituted with a phenyl group, said phenyl group being optionally substituted in meta or para position; Y and Z represent a hydrogen atom when X represents a carbon atom or Y and Z represent together an ether, thioether or amine bond optionally substituted with an alkyl or aryl group; R.sub.1 and R.sub.2 independently represent each H, Cl, F, I, Br and can be identical or different or R.sub.1 and R.sub.2 together represent a substituted or non-substituted fused benzene ring, R.sub.3 and R.sub.4 independently represent each H, Cl, F, I, Br and can be identical or different; R represents a group such that the O--R bond is capable of being hydrolysed by an enzyme. The invention also concerns a method for synthesizing said substrates, a composition, a kit and a method for detecting at least one micro-organism using said enzymatic substrate.  
SUMM The present invention also extends to a diagnostic kit comprising a composition as defined above and a container for the reaction medium. The term "container" is intended to mean any solid support, such as a bottle, a tube, a dish, a microtitration plate or a consumable product for an automatic machine, for instance API galleries or VITEK cards (registered trade marks, BioMerieux, Marcy l'Etoile, France).  
DETD The acetone is removed under reduced pressure and the residual solution is run into 300 ml of a solution of sodium carbonate at 0.06 mol/l at a

temperature of 0° C. with stirring. The chestnut brown precipitate is filtered under vacuum, washed with water and air-dried. The solid is dissolved in 100 ml of dichloromethane and washed thoroughly with a solution of potassium hydroxide at 0° C. in order to remove the excess p-naphtholbenzein. The residual p-naphtholbenzein is removed by stirring on Dowex Marathon resin in 100 ml of water at pH 11, for 2 to 3 hours. This purification is followed by thin-layer chromatography using an ethyl acetate/toluene (3:1) solvent, with developing using aqueous ammonia. The dark yellow solution is dried overnight on anhydrous magnesium sulfate, evaporated under reduced pressure, reconstituted with methanol and then re-evaporated so as to obtain a foam. This foam is dissolved in 50 ml of methanol and the product is deprotected for 5 hours using 20 ml of sodium methoxide in methanol at 0.4 mol/l. The solution is then adjusted to pH 6.5 using an IR120 (H.sup.+) resin and separated by settling out, and the solvent is removed under reduced pressure. The glycoside formed, p-naphtholbenzein- $\beta$ -galactosidase, (1.5 g) is in the form of a yellow-chestnut brown powder.

CLM What is claimed is:

19. A diagnostic kit comprising a composition as claimed in claim 16 and a container for the reaction medium.

IT 145-50-6, p-Naphtholbenzein

(preparation of aryl glycosides as enzyme substrates via coupling of p-naphtholbenzein with  $\alpha$ -acetobromogalactose)

LS ANSWER 2 OF 4 USPATFULL on STN

AN 2006:3879 USPATFULL

TI One-step enzymatic and amine detection technique

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PA Kimberly-Clark Worldwide, Inc. (U.S. corporation)

PI US 2006003336 A1 20060105

AI US 2004-881010 A1 20040630 (10)

DT Utility

FS APPLICATION

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CLMN Number of Claims: 33

ECL Exemplary Claim: 1

DRWN 3 Drawing Page(s)

LN.CNT 1794

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A technique for detecting the presence or quantity of an enzyme (or enzyme inhibitor) and/or an amine within a test sample is provided. For example, in one embodiment, a diagnostic test kit is employed that utilizes reactive complexes that each includes a substrate joined (e.g., covalently bonded, physically adsorbed, etc.) to a reporter and a separation species. Upon contacting the reactive complexes, enzymes may cleave the substrate and release the reporter. Moreover, the test kit may also employ a chemichromic dye, i.e., a dye that exhibits a detectable color change upon chemical reaction with one or more functional groups, such as amino groups. The signal generated (directly or indirectly) by the reporter and chemichromic dye may then be used to indicate the presence or quantity of an enzyme (or enzyme inhibitor) and amine, respectively, within the test sample.

SUMM In accordance with one embodiment of the present invention, a diagnostic test kit for detecting an amine, enzyme, or enzyme inhibitor within a test sample (e.g., vaginal fluid) is disclosed. The kit comprises a plurality of reactive complexes that each comprises a substrate joined to a reporter and a separation species. The substrate is cleavable by an enzyme to release the reporter. The kit further comprises a chromatographic medium that defines a first enzyme detection zone within which an enzyme detection signal is

capable of being generated. The presence or quantity of an enzyme, or an inhibitor thereof, is determinable from the enzyme detection signal. The chromatographic medium further defines an amine detection zone within which is contained a chemichromic dye. The chemichromic dye is capable of undergoing a color change in the presence of an amine, wherein the presence or quantity of an amine is determinable from the color change.

DRWD FIG. 1 is a perspective view of one embodiment of an assay device that may be used in the diagnostic test kit of the present invention;

DETD In general, the present invention is directed to a technique for detecting the presence or quantity of an enzyme (or enzyme inhibitor) and/or an amine within a test sample. For example, in one embodiment, a diagnostic test kit is employed that utilizes reactive complexes that each includes a substrate joined (e.g., covalently bonded, physically adsorbed, etc.) to a reporter and a separation species. Upon contacting the reactive complexes, enzymes may cleave the substrate and release the reporter. Moreover, the test kit may also employ a chemichromic dye, i.e., a dye that exhibits a detectable color change upon chemical reaction with one or more functional groups, such as amino groups. The signal generated (directly or indirectly) by the reporter and chemichromic dye may then be used to indicate the presence or quantity of an enzyme (or enzyme inhibitor) and amine, respectively, within the test sample.

DETD As stated above, various separation techniques may be utilized in the present invention for separating any released reporters from unreacted complexes including, but not limited to, chemical separation techniques, magnetic separation techniques, etc. In one particular embodiment, for example, the diagnostic test kit contains an assay device that employs a chromatographic medium for separating unreacted complexes from released reporters. In contrast to other techniques, such as centrifugation, the use of a chromatographic medium may simplify and reduce the costs of the resulting diagnostic test kit for many consumer applications, including those in which a disposable kit is desired. Further, the use of a chromatographic medium also provides for a mechanism in which two different species, i.e., an enzyme (or inhibitor) and amine, may be simultaneously tested in a single step. That is, a user may use the kit to test a single sample for an enzyme (or inhibitor) and/or amine.

DETD Triarylmethane dyes, for example, may have the following general structure: ##STR2## wherein R, R', and R" are independently selected from substituted and unsubstituted aryl groups, such as phenyl, naphthyl, anthracenyl, etc. The aryl groups may, for example, be substituted with functional groups, such as amino, hydroxyl, carbonyl, carboxyl, sulfonic, alkyl, and/or other known functional groups. When contacted with the dye, the amino group of the amine (e.g., ammonia, diamines, and/or tertiary amines) reacts with the central carbon atom of the dye. The addition of the amino group causes the dye to undergo a change in color. An example of the resulting structure is set forth below: ##STR3##

DETD Besides diagnosing one or more types of infection in vaginal fluid, the method and diagnostic kit of the present invention may be used in any other application in which the detection of an enzyme, enzyme inhibitor, and/or amine may be desired. For example, many people (e.g., diabetics, burn victims, those suffering from suppressed immune systems, etc.) who have difficulty in healing and require extended periods for proper and complete wound healing are susceptible to infection. Bacteria and mold may also cause infection in hosts other than the human body, such as food. In many cases, these infections result in the formation of odorous amines and diamines, which may be produced by the metabolic processes of proteolytic bacteria together with short chain organic acids. Thus, as with vaginal infections, the ability to detect amines in other types contexts, such as in a wound exudate or food, may prove vastly beneficial. Likewise, the mere presence of an enzyme may, in some

cases, indicate the existence of tissue or organ damage. Abnormal enzyme concentrations may also indicate other conditions, such as a bacterial or viral infection. For instance, the presence or concentration of an enzyme in a test sample may also serve as a diagnostic marker for some types of cancers and other conditions. As an example, prostate-specific antigen (PSA) is a well-known marker for prostate cancer. Other examples of diagnostic markers include cathepsin B (cancer), cathepsin G (emphysema, rheumatoid arthritis, inflammation), plasminogen activator (thrombosis, chronic inflammation, cancer), and urokinase (cancer).

CLM What is claimed is:

1. A diagnostic kit for detecting an amine, enzyme, or an enzyme inhibitor within a test sample, the kit comprising: a plurality of reactive complexes that each comprises a substrate joined to a reporter and a separation species, said substrate being cleavable by an enzyme to release said reporter; and a chromatographic medium that defines a first enzyme detection zone within which an enzyme detection signal is capable of being generated, wherein the presence or quantity of an enzyme, or an inhibitor thereof, is determinable from said enzyme detection signal, said chromatographic medium further defining an amine detection zone within which is contained a chemichromic dye, said chemichromic dye being capable of undergoing a color change in the presence of an amine, wherein the presence or quantity of an amine is determinable from said color change.
2. A diagnostic test kit as defined in claim 1, wherein the enzyme is a protease or peptidase.
3. A diagnostic test kit as defined in claim 1, wherein said substrate is a protein, glycoprotein, peptide, nucleic acid, carbohydrate, lipid, ester, or derivative thereof.
4. A diagnostic test kit as defined in claim 1, wherein said substrate is casein, albumin, hemoglobin, myoglobin, keratin, gelatin, insulin, proteoglycan, fibronectin, laminin, collagen, elastin, or a derivative thereof.
5. A diagnostic test kit as defined in claim 1, wherein said reporter comprises a detectable substance that is capable of directly generating said enzyme detection signal.
6. A diagnostic test kit as defined in claim 1, wherein said reporter comprises a specific binding member.
7. A diagnostic test kit as defined in claim 6, further comprising probes conjugated with a specific binding member, said probes comprising a detectable substance that is capable of directly generating said enzyme detection signal.
8. A diagnostic test kit as defined in claim 1, wherein said separation species is a specific binding member.
9. A diagnostic test kit as defined in claim 8, wherein a receptive material is immobilized within said first enzyme detection zone that has an affinity for said specific binding member.
10. A diagnostic test kit as defined in claim 1, wherein said separation species is a magnetic particle.
11. A diagnostic test kit as defined in claim 10, further comprising a magnetic device positioned adjacent to said chromatographic medium to immobilize said magnetic particle within a separation zone.
12. A diagnostic test kit as defined in claim 1, wherein said chromatographic medium further comprises a second enzyme detection zone

within which a second enzyme detection signal is capable of being generated.

13. A diagnostic test kit as defined in claim 12, wherein a second receptive material is immobilized within said second detection zone that is capable of binding to said reporter or complexes thereof to generate said second enzyme detection signal.

14. A diagnostic test kit as defined in claim 12, wherein a second receptive material is immobilized within said second detection zone that is capable of binding to probes or complexes thereof to generate said second enzyme detection signal.

15. A diagnostic test kit as defined in claim 1, wherein said chemichromic dye is an arylmethane.

16. A diagnostic test kit as defined in claim 1, wherein said chemichromic dye is a triarylmethane having the following general structure: ##STR9## wherein R, R', and R" are independently selected from substituted and unsubstituted aryl groups.

17. A diagnostic test kit as defined in claim 1, wherein said chemichromic dye is a diarylmethane.

18. A diagnostic test kit as defined in claim 1, wherein said amine detection zone is positioned downstream from said first enzyme detection zone.

19. A diagnostic kit for detecting an amine or a hydrolytic enzyme within a test sample, the kit comprising: a plurality of reactive complexes that each comprises a substrate joined to a reporter and a specific binding member, said substrate being cleavable by a hydrolytic enzyme to release said reporter; and a chromatographic medium that defines a first enzyme detection zone within which an enzyme detection signal is capable of being generated, wherein the presence or quantity of a hydrolytic enzyme is determinable from said enzyme detection signal, said chromatographic medium further defining an amine detection zone positioned downstream from said first enzyme detection zone, wherein a chemichromic dye is contained within said amine detection zone, said chemichromic dye being capable of undergoing a color change in the presence of an amine, wherein the presence or quantity of an amine is determinable from said color change.

20. A diagnostic test kit as defined in claim 19, wherein said reporter comprises a detectable substance that is capable of directly generating said enzyme detection signal.

21. A diagnostic test kit as defined in claim 19, wherein said reporter comprises a specific binding member.

22. A diagnostic test kit as defined in claim 21, further comprising probes conjugated with a specific binding member, said probes comprising a detectable substance that is capable of directly generating said enzyme detection signal.

23. A diagnostic test kit as defined in claim 19, wherein said chromatographic medium further comprises a second enzyme detection zone within which a second enzyme detection signal is capable of being generated.

24. A diagnostic test kit as defined in claim 23, wherein said second detection zone is capable of capturing said reporter or complexes thereof to generate said second enzyme detection signal.

25. A diagnostic test kit as defined in claim 19, wherein said chemichromic dye is a triarylmethane having the following general structure: ##STR10## wherein R, R', and R" are independently selected from substituted and unsubstituted aryl groups.

26. A diagnostic test kit as defined in claim 19, wherein said chemichromic dye is a diarylmethane.

IT 145-50-6,  $\alpha$ -Naphtholbenzein  
(stripe on nitrocellulose membrane in amine detection zone; diagnostic kit and one-step enzymic and amine detection technique using enzyme substrate joined to reporter and separation species and chromatog. medium with detection zones)

L5 ANSWER 3 OF 4 USPATFULL on STN

AN 2005:130627 USPATFULL

TI Odor controlling article including a visual indicating device for monitoring odor absorption

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PI US 2005112085 A1 20050526

AI US 2003-687269 A1 20031016 (10)

DT Utility

FS APPLICATION

LREP DORITY & MANNING, P.A., POST OFFICE BOX 1449, GREENVILLE, SC, 29602-1449, US

CLMN Number of Claims: 18

ECL Exemplary Claim: 1

DRWN 3 Drawing Page(s)

LN.CNT 827

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

SUMM The visual indicating agent is typically color-sensitive to at least one odors selected from the group comprising body odor, foot odor, garbage odor, urinary odor, feces odor, tobacco odor, raw meat odor, other common household odors such as bathroom, pet and cooking odors, mercaptans (or thiols), amines, ammonia, sulfur, sulfides, hydrogen sulphide, sulfur degradation products, aliphatic acids, isovaleric acid, butyric acid, and acetic acid.

SUMM where R, R' and R" are as shown in Table 1:

TABLE 1

Indicating agents having the general formula (I) or (II)

Indicating Agent	R	R'	R"
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Indicating Agent for

Michler's Hydrol H (CH.sub.3).sub.2NC.sub.6H.sub.5--  
(CH.sub.3).sub.2NC.sub.6H.sub.5-- Thiols, Mercaptans,

(MH)

Ammonia, Amines,

Diamines and Polyamines

Pararosaniline (NH.sub.2)C.sub.6H.sub.5-- (NH.sub.2)C.sub.6H.sub.5--

(NH.sub.2)C.sub.6H.sub.5-- Ammonia, Amines,

Base (PAB)

Diamines and Polyamines

Alpha-naphtholbenzene (ANB) C.sub.6H.sub.5-- ##STR2##

##STR3##

Ammonia, Amines, Diamines and

Polyamines

SUMM In some instances, the visual indicating agent and odor absorbing agent may be the same agent. For example, BDMB may be used as both the odor absorbing agent and the visual indicating agent for sulfur, amine and ammonia odors.

DRWD FIG. 2 shows a standard curve for the detection of ammonia by BDMB;

DETD For example, the generation of odor from urine is mostly based on chemical and biological degradation of urine components, and amines, ammonia and sulfur degradation products (methyl mercaptan and hydrogen sulfide) are the major odor sources in urine. They can also be found in feces odor and body odor. Additionally, enzymes such as urease can convert urea, a major component in urine, to ammonia and thereby increase the generation of odors in urine. Aliphatic acids such as valeric, isovaleric, butyric and acetic acids are commonly found to be the major odor components in body odors, foot odor, tobacco smoke, raw meat, garbage (kitchen) odor, cat odor and the musty smell of basements and cellars. Table 2 shows the concentration of the chemical components of common household odors along with their human threshold values (concentration that can be detected by the human nose).

TABLE 2

Concentration of the chemical components of common household odors along with their human threshold values

Odor	Major Chemical Components	Concentration (ppb)	Human Threshold (ppb)
Dog odor	Ammonia	5900	1500
	Trimethylamine	1500	0.03
	Methyl mercaptan	0.5	0.07
Cat odor	n-Butyric acid	0.3	0.19
	Ammonia	1980	1500
	Trimethylamine	0.3	0.03
Garbage odor	Methyl mercaptan	56	0.07
	n-Butyric acid	166	0.19
	n-Valeric acid	52	0.04
Sock odor (foot)	iso-Valeric acid	1.36	0.08
	n-Valeric acid	0.1	0.04
	Hydrogen sulfide	0.7	0.41
Cooking odor - Fish	Acetaldehyde	1740	1.50
	Dimethyl sulfide	41	3.0
	Hydrogen sulfide	11	0.41
Cooking odor - Shrimp	Acetaldehyde	15	1.5
	trimethylamine	96	0.03
	Hydrogen sulfide	11	0.41
Cooking odor - Chicken breast	Carbon disulfide	283	0.5
Bathroom odor - Urine	Methyl mercaptan	0.07	0.06
Bathroom odor - Feces	Hydrogen sulfide	32	0.41
	Hydrogen sulfide	32	0.41
	Methyl mercaptan	2.3	0.07
	Acetic acid	29.7	6.0

DETD In the examples which follow, several color changing indicating agents that are sensitive to very low levels (for example >0.01 parts per billion (ppb), more preferably from >10 ppb, and most preferably >100 ppb) of amines, ammonia, sulfur compounds, carboxylic acids and aldehydes were identified (Table 3). While the indicating agent may not detect the lower levels of odorous compounds immediately, it may change color in response to these low levels over a period of time,

which may be hours (for example, in the case of a diaper), days, weeks or even months (such as in an air filter). The indicating agents are all available from Aldrich Chemical Co. of Milwaukee, Wis.

DETD The quantity of odor absorbing agent used in the odor absorbing article will depend on the nature of the article and amount of odor it is intended to absorb, and will therefore vary from article to article. For example, a disposable diaper which is intended to absorb urine and feces odors may contain a different amount of odor to a sheet intended to absorb pet odor over a longer period of time. By measuring the odor absorption capacity of the sheet or article (mg odor absorbable/gram of sheet) and knowing that the indicating agent reacts with the odor compound (mole of odor compound/mole of indicating agent), the odor absorption capacities can be matched to tune the indicating agent to the odor absorption of the sheet or article. Thus, without intending to limit the invention in any way, the indicating agent may be present in an amount of from 0.001 to 15% wt/wt, more preferably from 0.005 to 5% wt/wt, and most preferably from 0.1 to 1% w/wt. As the amount of indicating agent used in the invention will depend on the amount of odor which can be absorbed by the article, the concentration of indicating agent which is applied to the article will also vary according to the article.

TABLE 3

Visual indicating agents and the specific odors that cause color change  
Visual Indicating Agent                      Odor or Odor Class

Michler's Hydrol	Ammonia, amines, sulfur compounds
Copper salts and complexes	Ammonia, amines, sulfur compounds
Rose Bengal (Acid Red 94)	Sulfur compounds
D&C Red 28 (Acid Red 92)	Sulfur compounds
Cobalt salts and complexes	Sulfur compounds, aldehydes, amines
Copper phenanthroline	Sulfur compounds and amines
Iron salts and complexes	Sulfur compounds and amines
Phenol red	Aliphatic carboxylic acids
Cresol red	Aliphatic carboxylic acids
Neutral red	Aliphatic carboxylic acids
3-Nitrophenol	Aliphatic carboxylic acids
Brilliant Yellow	Aliphatic carboxylic acids
Bromothymol blue	Aliphatic carboxylic acids
Chlorophenol red	Aliphatic carboxylic acids
Pararosaniline base	Ammonia and amines
Alpha-naphtholbenzene	Ammonia and amines
Naphthochrome green	Ammonia and amines

DETD As shown in FIG. 2, a standard curve was derived using ammonium hydroxide solution as an ammonia odor source detected by BDMB (MH-dye). In FIG. 2 the x-axis is the concentration of ammonia in ppb from 0 to 400 and the y-axis is the absorbance at 590 nm. Into each of 8 vials, 50  $\mu$ l of a specific concentration of ammonia solution (0, 0.01, 0.02, 0.04, 0.08, 0.16, 0.32, and 0.64%, respectively) was mixed with 150  $\mu$ l of MH solution (20  $\mu$ l of 10.0 mg/ml MH in CH.sub.3CN with 5.0 ml of 40 mM sodium acetate and 4 M guanidine HCl, pH 5.1), all available from Aldridge Chem. Co. of Milwaukee, Wis. and the vials were sealed and incubated for less than 4 min.

DETD The solutions were then transferred to microtiter plate wells and the absorbances were measured at 590 nm using the microtiter plate reader from Dynex Technologies of Chantilly, Va. (Model # MRX). The absorbance readings were plotted against the concentrations of ammonia solutions, with the concentrations being represented as parts per billion (ppb). The sensitivity of ammonia detection was very high according to the MH-dye method, and it was shown that the sensitivity could be altered by varying the MH-dye concentration.

DETD It was therefore concluded that BDMB is an effective, multi-functional



odor reducing agent for sulfur, amine and ammonia odors which are major components of, among others, urine, feces, dog and cooking odors.

CLM What is claimed is:

10. The article of claim 1, wherein the odor is selected from the group consisting of body odor, foot odor, urinary odor, tobacco odor, meat odor, garbage odor, basement odor, mercaptans, sulfide, hydrogen sulfide, amines, ammonia, sulfur, sulfur degradation products, aliphatic acids, isovaleric acid, butyric acid and acetic acid.

IT 51-28-5, 2,4-Dinitrophenol, uses 72-48-0, Alizarin 76-59-5, Bromothymol blue 76-60-8, Bromocresol green 76-61-9, Thymol blue 115-39-9, Bromophenol blue 119-58-4, Michler's hydrol 125-20-2, Thymolphthalein 143-74-8, Phenol red 145-50-6 467-62-9 493-52-7, Methyl red 547-58-0, Methyl orange 548-62-9, Crystal violet 554-84-7, m-Nitrophenol 596-27-0, o-Cresolphthalein 1733-12-6, Cresol red 2650-18-2, Calcocid Blue 2G 4430-20-0, Chlorophenol red 4474-24-2, Acid Blue 80 5715-76-4 6441-64-1, Brilliant Yellow 8004-87-3, Methyl violet 16423-68-0, Erythrosine B 87831-33-2, Ethyl Red 851040-24-9, Bromocresol mauve  
(as indicator; odor controlling article including visual indicating device for monitoring odor absorption)

L5 ANSWER 4 OF 4 USPATFULL on STN

AN 2005:99061 USPATFULL

TI Method and device for detecting ammonia odors and helicobacter pylori urease infection

IN Boga, RameshBabu, Roswell, GA, UNITED STATES

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PI US 2005084977 A1 20050421

AI US 2003-687327 A1 20031016 (10)

DT Utility

FS APPLICATION

LREP DORITY & MANNING, P.A., POST OFFICE BOX 1449, GREENVILLE, SC, 29602-1449, US

CLMN Number of Claims: 26

ECL Exemplary Claim: 1

DRWN 4 Drawing Page(s)

LN.CNT 589

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

TI Method and device for detecting ammonia odors and helicobacter pylori urease infection

AB The invention provides a breath testing device which visually indicates the presence of ammonia in a patient's breath, in particular ammonia from helicobacter pylori urease infection. The breath testing device comprises a visual indicating agent which changes color in response to ammonia odors, such as 4,4'-bis(dimethylamino)-benzhydrol (Michler's hydrol or BDMB), pararosaniline base and alpha-naphtholbenzein. The indicating agent is applied to a substrate which is then inserted into a tube or straw, which can be attached to the inlet of a breath collection balloon. When the patient blows into the tube or straw, the indicating agent will change color if it detects levels of ammonia which are consistent with helicobacter pylori urease infection.

SUMM The present invention relates to a method and device for detecting ammonia odors and uses thereof, in particular for diagnosing helicobacter pylori urease infection.

SUMM It is estimated that almost 1 in 10 adults will develop a stomach ulcer at some time in their lives. The bacterium Helicobacter pylori (HP) is known to be the cause of most stomach ulcers and ulcers of the duodenum and, once detected, can be cured by treatment with antibiotics. H. pylon (HP) produces an active form of the urease enzyme, which hydrolyzes urea

into ammonia and carbon dioxide. Since ammonia is the key component generated by urease-catalyzed hydrolysis of urea, it follows that the presence of this compound in a person's breath can be used to diagnose helicobacter pylori urease (HPU) infection.

SUMM However, there are not many systems to detect ammonia odors, and of those available, most require the use of expensive instrumentation and are complex in operation (and hence not suitable for use by untrained users).

SUMM In general, HPU infection is diagnosed by analyzing the difference of exhaled ammonia and/or CO<sub>2</sub> levels from a patient before and after swallowing a dose of radioactive urea. The levels of ammonia in HPU infected persons are usually between 100 to 200 parts per million (ppm) after swallowing the urea, whereas the levels of ammonia in an uninfected person after having swallowed the urea are much the same as before taking the urea (<2 ppm).

SUMM Therefore, there is a clear need for a simple, safe device which needs neither expensive instrument nor radioactive materials for detecting ammonia from HPU infection and other sources.

SUMM This invention describes a simple device for detecting ammonia odors using a visual indicating agent which changes color when ammonia is present in the breath of a user, in particular when the ammonia is present in the range of 20 to 500 parts per million (ppm), more preferably in the range of 50 to 400 ppm, and most preferably in the range of 75 to 300 ppm.

SUMM The indicating agent is also suitable for diagnosing helicobacter pylori urease (HPU) infection, which causes ammonia to be present in a patient with the infection. The development of potential stomach ulcers can thus be inhibited or the stomach ulcers can be treated at an early stage.

SUMM The visual indicating agent is typically a dye which is color sensitive to ammonia odors, such as 4,4'-bis(dimethylamino)-benzhydrol (BDMB or Michler's hydrol (MH)), a dye having a similar chemical structure to MH, a triamino-triphenyl-methanol dye such as pararosaniline base (PAB), alpha-naphtholbenzein or any other dye which has high sensitivity for ammonia. The dye may change color by fading to a lighter color, by deepening in color or by actually changing from one color to another.

SUMM In both embodiments, when the patient blows into the tube, their breath will pass through the tube and over or through the substrate, thus causing the indicating agent to change color if levels of ammonia which are indicative of HPU infection (generally between 20 and 500 ppm) are present in the patient's breath.

SUMM The straw may be connected to the inlet of a breath collection balloon such as the type sold commercially by Kimberly-Clark/Ballard Medical Devices of Draper, Utah for use in the existing H. pylori detection kits (PYtest.TM. 14C-Urea Breath Test). The use of such a balloon would help ensure that the correct amount of breath was sampled, as the patient would simply blow through the straw into the balloon until it was fully inflated.

SUMM The patient may ingest urea prior to his or her breath being tested so as to boost the ammonia levels which are detected. After a period of time sufficient to allow HPU, if present, to hydrolyze the urea into ammonia and carbon dioxide, the patient would then blow into a breath testing device. If the patient were infected with HPU, sufficient amounts of ammonia would be present in his or

her breath to be detected by the device, and the indicating agent would show a change in color.

- DRWD FIG. 1 shows a standard curve for the detection of ammonia by Michler's Hydrol-dye;
- DRWD FIG. 2 shows a standard curve for the detection of ammonia by pararosaniline base (PAB);
- DRWD FIG. 3 shows the results of a test to monitor the hydrolysis of urea to ammonia and carbon dioxide using PAB;
- DRWD FIG. 4 shows simple breath testing device according to a first embodiment of the invention in an unassembled state, demonstrating a color change in the indicating agent before (left) and after (right) exposure to ammonia odors;
- DRWD FIG. 5 shows a second embodiment of a breath testing device according to the invention with a color reference, before (right) and after (left) exposure to ammonia odors;
- DETD The invention provides simple breath testing devices which are able to detect levels of ammonia odors in a patient's breath which are consistent with helicobacter pylori urease infection without requiring the patient to ingest radio-active chemicals. Thus, the breath testing devices include a visual indicating agent which changes color in response to the ammonia odors, preferably when the ammonia is in the range of 20 to 500 parts per million (ppm), more preferably in the range of 50 to 400 ppm, and most preferably in the range of 75 to 300 ppm.
- DETD Michler's hydrol (MH) and related indicating agents, which are also suitable for use as indicating agents for the present invention, can be represented by the following general formula (I) or (II):

##STR2##

Indicating Agent R R' R"  
Indicating Agent for

Michler's Hydrol H (CH.sub.3).sub.2NC.sub.6H.sub.5--  
(CH.sub.3).sub.2NC.sub.6H.sub.5-- Ammonia, Amines,  
(MH)

Diamines and Polyamines  
Pararosaniline (NH.sub.2)C.sub.6H.sub.5-- (NH.sub.2)C.sub.6H.sub.5--  
(NH.sub.2)C.sub.6H.sub.5-- Ammonia, Amines,  
Base (PAB)  
Diamines and Polyamines

Alpha-naphtholbenzein (ANB) C.sub.6H.sub.5-- ##STR3##  
##STR4## Ammonia, Amines, Diamines and  
Polyamines

Naphthochrome Green (NCG) C.sub.6H.sub.5-- ##STR5## ##STR6##  
Ammonia, Amines, Diamines and Polyamines

DETD The dye may change color by fading to a lighter color, by deepening in color or by actually changing from one color to another. Thus, MH changes from blue to colorless, PAB changes from red to colorless and ANB changes from yellow/orange to grey in the presence of ammonia.

DETD The degree of the color change will depend on the concentration of the indicating agent or the concentration of ammonia in the patient's breath. Therefore, in order to observe a color change in response to ammonia levels in the range of 75 to 300 ppm, the concentration of indicating agent which is used is preferably in the range of from 0.01 to 15% wt/wt, more preferably from 0.05 to 2% wt/wt, and most preferably from 0.1 to 0.5% wt/wt.

DETD In both embodiments, when the patient blows into the tube, their breath

will pass through the tube and over or through the substrate, thus causing the indicating agent to change color if levels of ammonia which are indicative of HPU infection (generally from 20 to 500 ppm, more particularly from 50 to 400 ppm and most particularly between 75 to 300 ppm) are present in the patient's breath.

DETD The straw or tube may be connected to the inlet of a breath collection balloon such as the type sold commercially by Kimberly-Clark/Ballard Medical Devices of Draper, Utah for use in the existing H. pylori detection kits (PYtest.TM. 14C-Urea Breath Test). Such a test balloon has a volume of about 0.5 to 2 liters or about 1 lung-full of breath for most individuals. The use of such a balloon helps ensure that the correct amount of breath is sampled. If too much breath is sampled, the device may provide a false positive diagnosis, and if too little breath is sampled, the device may provide a false negative diagnosis. Thus, the patient would simply blow through the straw into the balloon until it was fully inflated.

DETD The patient may ingest urea prior to his or her breath being tested so as to boost the ammonia levels which are detected. After a period of time sufficient to allow HPU, if present, to hydrolyze the urea into ammonia and carbon dioxide, the patient would then blow into the breath testing device. If the patient were infected with HPU, sufficient amounts of ammonia would be present in his or her breath to be detected by the device, and the indicating agent would show a change in color.

DETD A reaction mixture was placed into each of 8 vials containing: 50  $\mu$ l of ammonia hydroxide solution as an ammonia source (0, 0.01, 0.02, 0.04, 0.08, 0.16 and 0.64% of ammonia hydroxide, respectively) and 150  $\mu$ l of MH dye (20  $\mu$ l of 10.0 mg/ml MH in CH.sub.3CN with 5.0 ml of 40 mM sodium acetate and 4M guanidine HCl, pH5.1). After incubation of all the vials at room temperature for less than 4 minutes, a 200  $\mu$ l portion from each vial was transferred to a microtiter plate well, and the absorbances were measured at 590 nm using a microtiter plate reader (The absorbances can also be measured in the range of 580-615 nm).

DETD As shown in FIG. 1, a standard curve was derived by plotting the absorbance readings against the concentrations (ppb) of ammonia solutions. In FIG. 1, the x-axis is the concentration of ammonia in parts per billion (ppb) from 10 to 400 and the y-axis is the absorbance at 590 nm from 1 to 0.7. The sensitivity of ammonia detection by MH was shown to be very high.

DETD A similar study was carried out with another dye, pararosaniline base (PAB), which was shown to be sensitive to amine and ammonia odors. In order to generate a standard curve (FIG. 2), a reaction mixture was placed into each of 8 vials containing 50  $\mu$ l of an ammonia hydroxide solution as an ammonia source (0, 0.01, 0.02, 0.04, 0.08, 0.16 and 0.64% of ammonia hydroxide, respectively) and 150  $\mu$ l of PAB solution (10  $\mu$ l of 10 mg/ml PAB stock solution made in CH.sub.3CN with 5.0 ml of 40 mM sodium acetate and 4 M guanidine HCl, pH5.1). 200  $\mu$ l of each reaction mixture was transferred to a microtiter plate well and the wells were incubated at room temperature for 4 to 5 min. The absorbances were then read at 550 nm using a microplate reader. PAB was shown to be highly selective for ammonia and amine odors. In FIG. 2, the x-axis is the concentration of ammonia in parts per billion (ppb) from 10 to 400 and the y-axis is the absorbance at 550 nm from 1 to 1.0.

DETD PAB was then used to see if it was suitable for use in monitoring the reaction in which urease catalyzes urea to ammonia and carbon dioxide by-products (FIG. 3). Into each of two vials (expt. 1 and expt. 2) was placed 1 ml of a reaction mixture containing 100  $\mu$ l of 10 mM urea, 850  $\mu$ l of 10 mM PBS, pH7.3, and 50  $\mu$ l of 10.0 mg/ml urease. Three control vials were prepared, the first control excluding both urea and urease (control 1), the second control excluding urease but containing urea (control 2), and the third control excluding urea but containing urease (control 3). The vials were vortexed and 50  $\mu$ l from

each vial was transferred to a microtiter plate well. PAB solution (10  $\mu$ l of 10 mg/ml PAB stock solution made in CH<sub>3</sub>sub.3CN with 5.0 ml of 40 mM sodium acetate and 4 M guanidine HCl, pH5.1) was then added to each well and the absorbance change with time was monitored at 550 nm using a microplate reader. In FIG. 3, the x-axis is time in minutes and the y-axis is the absorbance at 590 nm from 1 to 1.0. As can be seen in FIG. 3, the three controls had relative constant absorbance over time while the two experimental samples had falling absorbance over time. The PAB did not show any interference with buffer, urea, or urease alone, and was shown to be sensitive for ammonia generated by the urease reaction with urea.

DETD A first embodiment of a device 10 for detecting HPU infection was designed using an ammonia-odor sensitive dye coated on a cellulose substrate (FIG. 4). Accordingly, 1 mg/ml stock solution of MH-dye was applied onto a SCOTT® paper towel from Scott Paper of Mississauga, ON, Canada, that had been previously coated with a 1 weight percent (dry) solution of SNOWTEX-O® nanoparticles and allowed to air dry. The dye-coated paper towel was then cut into 2 cm+4 cm strips 12 which were rolled up and each strip 12 was inserted into a clear plastic drinking straw 14 from Glad Products Company of Oakland, Calif.

DETD The devices were tested by injecting known concentrations of ammonia hydroxide into the straws to determine their sensitivity to ammonia odors. A color change (from blue 16 to colorless 18) was noticed and was clearly visible in the presence of ammonia odors.

DETD The experiment was repeated using PAB-dye and alpha-naphtholbenzein dye instead of MH-dye. On exposure to ammonia odors, the dye-coated substrates were observed to change from red to colorless and from yellow/orange to grey, respectively.

DETD The color intensities (La\*b\*) of the indicating dye showed a clear difference between before and after the exposure to ammonia odors (.about.100 ppb). The level of detection of ammonia odor by either MH or PAB (-100 ppb) is far less compared to the physiological level generated from urea hydrolysis by HP urease, V.sub.max, 1,100 $\pm$ 200  $\mu$ mol of urea hydrolyzed/min/mg of protein.

DETD The ink solution was loaded into empty Margarita® cartridges (part no. 0900400-300) obtained from McDermid-Colorsplan of Eden Prairie, Minn. and printed using a wide format McDermid-Colorsplan printer (Model XII). A strip of the printed Scott® paper towel was then exposed to ammonia odor and the blue color was observed to decolorize in about 10 seconds (compared to 3-5 minutes taken to observe the color change of a Scott® paper towel saturated with MH according to one of the previous examples). Higher sensitivity to the odor was thus observed by inkjet printing the indicating agent onto the substrate.

DETD In order to ensure that the HPU testing devices as described above would detect the levels of ammonia which are exhaled by patients having ulcers, it is preferable that a uniform amount of breath is sampled. Thus, the device described in example 4 was attached to a breath collection balloon 50 from Kimberly-Clark/Ballard Medical Devices of Draper, Utah (FIG. 8), discussed above. The patient would therefore blow through the device 10 into the balloon until the balloon becomes inflated, and if the substrate 12 with the indicating agent within the device 10 changed color after exposure to this amount of breath, this would indicate that the patient is suffering from HPU infection.

DETD From the above examples, it can be seen that it is possible to detect HPU infection using a visual indicating agent which is sensitive to ammonia and/or amine odors, rather than requiring a patient to ingest radioactive materials and to use expensive and complex equipment.

CLM What is claimed is:

1. A breath testing device for detecting the presence of ammonia odors, comprising a visual indicating agent that is color sensitive to ammonia.

2. The breath testing device of claim 1, wherein the visual indicating agent is sensitive to ammonia levels which are present in the range of from 20 to 500 parts per million.

3. The breath testing device of claim 1, wherein the visual indicating agent is sensitive to ammonia levels which are present in the range of from 50 to 400 parts per million.

4. The breath testing device of claim 1, wherein the visual indicating agent is sensitive to ammonia levels which are present in the range of from 75 to 300 parts per million.

19. The breath testing device of claim 1, further comprising a zone having a reference color printed thereon, the reference color being the color to which the indicating agent will change when it is exposed to ammonia odors from helicobacter pylori urease infection.

21. A breath testing device comprising a carrier portion defining a passage which is open on at least one end, wherein said device has a visual indicating agent that is color sensitive to ammonia.

24. A kit for detecting helicobacter pylori urease infection which comprises a breath testing device having a visual indicating agent that is color sensitive to ammonia and a breath collecting device.

25. A method of testing for helicobacter pylori urease infection in a patient comprising the steps of: causing the patient to blow or breath into a carrier portion of a device containing a visual indicating agent that is sensitive to ammonia odors from helicobacter pylori urease infection; and observing if the visual indicating agent changes color to indicate that the patient is infected with helicobacter pylori urease.

IT 119-58-4, 4,4'-Bis(dimethylamino)-benzhydrol 145-50-6,  
α-Naphtholbenzein 548-61-8, Pararosaniline base  
(method and device for detecting ammonia odors and Helicobacter pylori  
urease infection)

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<input type="checkbox"/>	L15	\$naphtholbenzein	95
<input type="checkbox"/>	L16	\$naphtolbenzein	0
<input type="checkbox"/>	L17	\$benzylidene\$	26976
<input type="checkbox"/>	L18	\$hydroxybenzylidene\$	1133
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<input type="checkbox"/>	L22	para near magenta	42
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<input type="checkbox"/>	L24	("C.I.42500")!	5
<input type="checkbox"/>	L25	(CI42500)!	3
<input type="checkbox"/>	L26	(PARA-ROSANILINE   PARA-ROSANILIN   PARA-ROSANILNE   PARA- ROSANININE   PARA-ROSANI-LINE   PARA-ROSANILIN-DERIVATIVE   PARA-ROSANALINE   PARA-ROSALINE   PARA-ROSIIIILINE   PARA-	65



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☐ 1: IARC Monogr Eval Carcinog Risks Hum. 1993;57:215-34.

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**Magenta and CI Basic Red 9.**

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### Optical sensors for monitoring and control of plant growth systems.

**Tabacco MB, Zhou Q, DiGiuseppe TG.**

GEO-CENTERS, Newton Centre, MA 02159, USA.

Optical chemical sensors have been developed for monitoring several parameters relevant to plant growth systems. These sensors utilize porous polymer and porous glass as the sensing element, and optical fiber input/output lines connected to a custom optoelectronic interface. Present in the sensing element are immobilized colorimetric indicators, which react with the analyte to be sensed. This reaction results in a change in the optical properties of the sensor. These sensors are particularly suited to in-situ monitoring of nutrient solution parameters and atmospheric trace contaminants in life support and plant growth systems. Sensors for monitoring pH, ammonia, and ethylene will be discussed.

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Design and development of an automated and non-contact sensing system for continuous monitoring of plant health and growth. [Trans ASAE. 2001]

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: Appl Opt. 2002 Jan 20;41(3):573-8.



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**Transportable automated ammonia sensor based on a pulsed thermoelectrically cooled quantum-cascade distributed feedback laser.**

**Kosterev AA, Curl RF, Tittel FK, Kohler R, Gmachl C, Capasso F, Sivco DL, Cho AY.**

Rice Quantum Institute, Rice University, Houston, Texas 77251-1892, USA. akoster@rice.edu

A compact ammonia sensor based on a 10-microm single-frequency, thermoelectrically cooled, pulsed quantum-cascade laser with an embedded distributed feedback structure has been developed. To measure NH<sub>3</sub> concentrations, we scanned the laser over two absorption lines of its fundamental v<sub>2</sub> band. A sensitivity of better than 0.3 parts per million was achieved with just a 1-m optical path length. The sensor is computer controlled and automated to monitor NH<sub>3</sub> concentrations continuously for extended periods of time and to store data in the computer memory. c2002 Optical Society of America.

PMID: 11905584 [PubMed - indexed for MEDLINE]

Review: 0 ☐ 1: [ScientificWorldJournal](#). 2003 Jul 1;3:585-92.[LinkOut to  
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**Optical enzyme sensor for urea determination via immobilized pH indicator and urease onto transparent membranes.**

**Krysteva M, Al Hallak M.**

Department of Biotechnology, University of Chemical Technology and Metallurgy, Blvd. 8 Kl.Ohridski, 1756, Sofia. krysteva@uctm.edu

Transparent triacetylcellulose membranes with immobilized pH indicator (neutral red) as well as with simultaneously immobilized urease and neutral red were used as optical sensors for determination of urea concentrations in model solutions. Decomposition of urea with the enzyme urease is accompanied by evolution of ammonia. This leads to the changes of the neutral red absorption, which is proportional to the substrate (urea) within certain concentration limits in model solution. As a result of the investigation, standard curves were plotted for determination of urea over the range of 1 to 500 mM using immobilized indicator and free urease. Simultaneous immobilization of indicator and urease permitted determination of urea in the interval 50 to 500 mM. The membrane used contained 0.169 U urease activity on an area of 1.7 cm<sup>2</sup>. The standard curves were plotted using the linear region of the kinetic curves for the corresponding substrate concentrations. A possible scheme of the interaction between the activated triacetylcellulose membrane and the indicator and enzyme is proposed. The membranes obtained are suitable for repeated ecological applications where urea is to be determined

☐ 1: J Environ Monit. 1999 Oct;1(5):417-22.

Links

**Personal ammonia sensor for industrial environments.**

**Malins C, Doyle A, MacCraith BD, Kvasnik F, Landl M, Simon P, Kalvoda L, Lukas R, Pufler K, Babusik I.**

Department of Instrumentation and Analytical Science, UMIST, Manchester, UK M60 1QD. [chris.malins@umist.ac.uk](mailto:chris.malins@umist.ac.uk)

The realisation of an opto-chemical ammonia sensor suitable for personal monitoring tasks is described, comprising a cyanine dye immobilised in a microporous glass thin film. The fabrication of sensor platforms incorporating embossed grating couplers provides a compact optical design with effective waveguiding characteristics, resulting in reversible ammonia sensitivity in the 5-100 ppm range in under 2 min. Cross-sensitivity of sensor response with water and other potential interferents is considered.

PMID: 11529157 [PubMed - indexed for MEDLINE]

TABLE B

Other Indicators		
Indicator	Transformation Range, pH	Color Change
o-Cresol red (acid range)	0.2-1.8	Red-yellow
Thymol blue (acid range)	1.2-2.8	Red-yellow
Pentamethoxy red	1.2-3.2	Red violet-colorless
Tropeolin 00	1.3-3.2	Red-yellow
2,4-Dinitrophenol	2.4-4.0	Colorless-yellow
Methyl yellow	2.9-4.0	Red-yellow
Methyl orange	3.1-4.4	Red-orange
Tetrabromophenol blue	3.0-4.6	Yellow-blue
Alizarin sodium sulfonate	3.7-5.2	Yellow-violet
$\alpha$ -Naphthylred	3.7-5.0	Red-yellow
p-Ethoxychrysoidine	3.5-5.5	Red-yellow
Methyl red	4.4-6.2	Red-yellow
Bromocresol purple	5.2-6.8	Yellow-purple
p-Nitrophenol	5.0-7.0	Colorless-yellow
Azolitmin	5.0-8.0	Red-blue
Neutral red	6.8-8.0	Red-yellow
Rosolic acid	6.8-8.0	Yellow-red
$\alpha$ -Naphtholphthalein	7.3-8.7	Rose-green
Tropeolin 000	7.6-8.9	Yellow-rose red
Phenolphthalein	8.0-10.0	Colorless-red
$\alpha$ -Naphtholbenzein	9.0-11.0	Yellow-blue
Thymolphthalein	9.4-10.6	Colorless-blue
Nile blue	10.1-11.1	Blue-red
Alizarin yellow	10.0-12.0	Yellow-lilac
Salicyl yellow	10.0-12.0	Yellow-orange brown
Diazo violet	10.1-12.0	Yellow-violet
Tropeolin 0	11.0-13.0	Yellow-orange brown
Nitramine	11.0-13.0	Colorless-orange brown
Poirrier's blue	11.0-13.0	Blue violet-pink
Trinitrobenzoic acid (indicator salt)	12.0-13.4	Colorless-orange red

Indicator solutions are available commercially, together with equipment (comparators, color charts, and artificial color standards) suitable for visual color matching with the aid of reference buffer solutions. Examples of commercially available indicators are given in Table C.

TABLE C

Commercially Available Indicators		
Indicator	Range, pH	Color Change
Acid cresol red	0.2-1.8	Red-yellow
Acid metacresol purple	1.2-2.8	Red-yellow
Acid thymol blue	1.2-2.8	Red-yellow
Bromophenol blue	3.0-4.6	Yellow-blue
Bromocresol green	3.8-5.4	Yellow-blue
Methyl red	4.4-6.0	Red-yellow
Chlorophenol red	5.2-6.8	Yellow-red
Bromocresol purple	5.2-6.8	Yellow-purple
Bromothymol blue	6.0-7.6	Yellow-blue
Phenol red	6.8-8.4	Yellow-red
Cresol red	7.2-8.8	Yellow-red
Metacresol purple	7.6-9.2	Yellow-purple
Thymol blue	8.0-9.6	Yellow-blue
Phthalein red	8.6-10.2	Yellow-red
Tolyl red	10.0-11.6	Red-yellow
Acyl red	10.0-11.6	Red-yellow
Parazo orange	11.0-12.6	Yellow-orange
Acyl blue	12.0-13.6	Red-blue
Benzo yellow	2.4-4.0	Red-yellow
Benzo red	4.4-7.6	Red-blue
Thymol red	8.0-11.2	Yellow-red
Long-range indicator	3.0-11.0	Red-violet

These indicators can also be employed in practicing this invention.

In addition, mixed indicators can be employed in the invention. Specifically, for acid-base titrations and for cer-

tain other specialized applications indicators with a transformation point are often useful. The so-called "mixed indicators," consisting of an acid-base indicator and a suitable dye, have been developed to meet this need. A familiar example is the methyl orange-xylene cyanole mixture, which has an easily detectable and sharp point of transformation at pH 3.8. The improvement in the sharpness results from the superposition of the (pH dependent) color of the indicator and the color of the dye. Another typical mixed indicator consists of a solution of 1 per cent phenolphthalein and 0.2 per cent methyl green.

Mixtures of two acid-base indicators extend the pH range that can be covered by a single indicator test solution. Such mixed indicators also can be employed in this invention.

This invention can also be carried out with "one-color" indicators, only one form of which (usually the alkaline species) absorbs light in the visible region of the spectrum. Examples of one-color indicators are listed in Table D, together with their colors and transformation ranges.

TABLE D

One-Color Indicators		
(Acid form colorless) Transformation		
Name	Range, pH	Alkaline color
Picric acid	0.1-1.3	Yellow
2,6-Dinitrophenol	1.7-4.4	Yellow
2,4-Dinitrophenol	2.4-4.0	Yellow
2,5-Dinitrophenol	4.0-5.8	Yellow
p-Nitrophenol	5.3-7.6	Yellow
m-Nitrophenol	6.4-8.8	Yellow
Phenolphthalein	8.2-9.8	Red
Salicyl yellow	10.0-12.0	Yellow

In general, about 0.008% to about 0.032% (w/v) of the pH indicator in the test solution (or reference buffer solution) is sufficient for a color change upon freezing of the test solution. Use of the pH indicator at a concentration of about 0.016% (w/v) was found to be acceptable in the Examples. When the indicator itself is only slightly soluble in water, it is often convenient to utilize the water-soluble salt form. The amount of the pH indicator employed should be sufficient for a visible color change with the unaided eye when the composition of the invention is frozen. There does not appear to be an advantage in using large excesses of the pH indicator to enhance performance of the composition or the freeze-thaw device of the invention.

In general, the composition of this invention will have a pH of about 4.5 to about 10. The pH indicator and buffer solution are matched to the pH desired for the composition of the invention. The pH of the composition can, in turn, be selected based upon the pH of the material being monitored for freezing or thawing. For example, if a biological material having a pH of about 6 to about 9 is to be monitored, the pH of the composition of the invention will preferably be within this range, and the pH indicator and buffer will be selected to operate within the chosen range. In a preferred embodiment of this invention, the pH of the composition of the invention will be preferably within  $\pm 2$  pH units of the pH of the material being monitored for freezing or thawing.

In choosing a suitable buffer system, one should not only consider the pH required, but also take into account the nature of the system in which it will be employed. The added substances must not form insoluble compounds or complexes or enter into other undesired side reactions with the medium.

DOCUMENT-IDENTIFIER: US 6589761 B1

TITLE: Method and apparatus for detecting bacteria

Detailed Description Text (17):

The indicators listed below comprise various groups of indicators. The groups are regular acid-base indicators, mixed indicators, screened indicators, universal indicators, pH sticks, litmus paper (embedded in gel or litmus in a gel), natural food indicators, fluorescent indicators, luminescent indicators, irreversible indicators and a miscellaneous group. Many of these indicators can exist either in a water soluble form or an alcohol based form. Indicators that have been found to work include but are not limited to: ACID-BASE INDICATORS: Acid Fuchsin (red-yellow) Alkali Blue 6B Solution (alcoholic blue-red titrations) Alizarin (yellow-red) Alizarin Red S (yellow-red) Alizarin Yellow R (yellow-red) Alizarin Complexone, dihydrate (yellow-red) Andrade indicator with agar (yellow-red) Anilinesulfonphthalein (colorless-yellow) Aniline yellow (colorless-yellow) Arsenazo (black/purple-light blue) Azolitmin (red-blue) Azo violet (red/brown-yellow) Benzopurpurine (violet-red) Bindschedlers green (green-yellow) 2,2'-Bipyridyl (orange-light yellow) 4,4'-Bis(2-amino-1-naphthylazo) 2,2-stilbenedisulfonic acid (purple-red) 4,4'-Bis(4-amino-1-naphthylazo) 2,2-stilbenedisulfonic acid (brown-red) Brilliant Green (yellow-green) Brilliant Orange (yellow-red) Brilliant Yellow (yellow-red) Bromocresol Green (yellow-blue) Bromocresol Purple (yellow-purple) Bromochlorophenol Blue (yellow-blue/violet) Bromophenol Blue (yellow-blue) Bromophenol Red (yellow-red) Bromopyrogallol Red (yellow-red) Bromothymol Blue (yellow-blue) Bromoxyleneol Blue (yellow-blue) Calcein/Fluorexon (orange-light yellow) Calconcarboxylic Acid (orange-yellow) Calmagite (dark brown-light yellow) Carboxyarsenazo III (dark brown-light blue/yellow) Chlorophenol Red (yellow-red) Clayton Red (yellow-red) Clayton Yellow (yellow-amber) Cochineal (orange-yellow) Congo Red (blue-red) o-Cresolphthalein (red-yellow) Cresol Purple (red-yellow) Cresol Red, 1st and 2nd range (red-yellow) Crystal Red (yellow-blue) Cresolphthalein (yellow-red) Crystal Violet (yellow-blue) Curcumin (Tumarcin) (yellow-red) Cyanide Acid Blue (blue-red) Debrisoquine sulfate 3,4-Dihydro-2-[1H]-isoquinoli (yellow-red) p-(2,4 Dihydroxyphenylazo) benzenesulfonic acid, sodium salt ((yellow-orange) p-Dimethylaminoazobenzene (red-yellow) 4-(N,N-Dimethylamino)azobenzene (red-yellow) N 2,n2-dimethylquanosine (red-yellow) 4-(4-Dimethylamino-1-naphthylazo)-3-methoxybenzenesulfonic acid (violet-yellow) 3-(4-Dimethylamino-1-naphthylazo)-4-methoxybenzene (violet-yellow) 2-(p-Dimethylaminophenylazo)pyridine (yellow-blue) Dimethylsulfonazo III (yellow-blue) Diphenylalanine-4-sulfonic acid barium salt (yellow-blue) Diphenyl-4-Sulfonic acid sodium salt (yellow-blue) N,N-Dimethyl-p-(m-tolylazo)aniline (red-yellow) 2,4-Dinitrophenol (colorless-yellow) 2,5-Dinitrophenol (colorless-yellow) 2,6-Dinitrophenol (colorless-yellow) Diphenol Purple (yellow-purple) 2-(2,4 Dinitrophenylazo)-1-naphthol-3,6-disulfonic acid, sodium salt (yellow-red) 6,8-Dinitro-2,4-(1H) quinazolinedione (colorless-yellow) Diphenylalanine (yellow-blue) Diphenylalanine-4-sulfonic acid sodium salt (yellow-blue) Eosin (pink-red/purple) Epsilon Blue (orange-violet) Erichrome Blue Black R (blue/black-violet) Erythrosin, disodium salt (orange-red) 4-(p-Ethoxyphenylazo)-m-phenylene-diamine monohydrochloride (orange-yellow) Ethyl bis(2,4-dimethylphenyl) ethanoate (colorless-blue) Ethyl Orange (red-yellow) Ethyl Red (colorless-red) Ethyl Violet (yellow-blue) Fast Sulphon Black F (black-light blue/yellow) Ferroin Solution (red-green) Fluorexon (yellow-blue) Hematoxylin (brown-yellow) 8-hydroxypyrene-1,3,6-trisulfonic acid (colorless-blue) Indigo Carmine (blue-yellow) 5,5'-Indigodisulfonic acid, disodium salt (blue-yellow) Indigo Trisulfonate Potassium Salt (blue-yellow) Indole Pentasodium (blue-yellow) Indolphenol Sodium Salt (blue-yellow) Leucocrystal violet (blue-yellow) Litmus (Azolitmin) (red-yellow) Malachite Green (yellow-blue/green) Metacresol Purple (red-purple) Metanil Yellow (red-yellow) Methylene Blue (deep green-blue) Methyl Green (yellow-blue) 4-Methylumbelliferone methyleneminiodiacetic acid (red-orange) Methyl Orange (red orange) Methyl Purple (red-orange) Methyl Red (red -yellow) Methylthymol Blue (green/brown-yellow) Methyl Violet (yellow-blue) Methyl Viologen (yellow-blue) Methyl Yellow (colorless-yellow) Murexide Powder

(deep red-yellow) 1-Naphthobenzein (yellow-green) o-Naphtholbenzein (yellow-green) p-Naphtholbenzein (yellow-blue) a-Naphthoflavone (yellow-blue) Naphtholphthalein (yellow-blue) 1-Naphthyl Red (yellow-blue) Neutral Red (red-yellow) Nitramine (colorless-orange/brown) Nitroaniline (yellow-green) Nitrazine Yellow Powder (yellow-green) 4-Nitrophenol (colorless-yellow) 3-Nitrophenol (colorless-yellow) p-Nitrophenol (colorless-yellow) Nitrosulfonazo III (colorless-yellow) Orange G (red-yellow) Orange 1 (Tropaeolin 000 No. 1) (rose-yellow) m-Orange IV (Tropaeolin 00) (red-yellow) Oregon Green/derivatives like Oregon Green carboxylic acid (deep green-yellow) Paramethyl Red (red-yellow) Patent Blue (deep blue-yellow) 1,10-Phenolanthronine (colorless-yellow) 5-Nitro-1,10-phenanthroline hydrate (colorless-pink) Phenolphthalein (colorless-pink) Phenolphthalin 2-[Bis(4-hydroxyphenylmethyl)]benzoic (colorless-pink) Phenol Red (yellow-red) Phenol Red Sodium Salt (yellow-red) Phenol Violet (yellow-blue) 4-Phenylazodiphenylamine (red-yellow) 4-Phenylazo-1-naphthylamine (red-yellow) Phloxine B (red/brown-yellow) Picric acid (yellow-colorless) Poirrier Blue (blue-red) Propyl Red (red-yellow) 1(2-Pyridylazo)-2-naphthol (red-yellow) Pyrocatechol Violet (red-yellow) Pyrogallol Red (deep yellow-colorless) Pyrogallolphthalein (pale-orange) Pyrogallosulfonphthalein (yellow-colorless) Quinoline Blue (colorless-blue) Quinaldine Red (colorless-red) Resazurin (red-blue) Resorcin Blue (red-blue) Resorcinol (red-blue) 4-(2-Pyridylazo)resorcinol monosodium salt (red-blue) 4-(2-Pyridylazo)resorcinol disodium salt (red-blue) Resorinol Yellow (Tropaeolin 0) (yellow-orange/brown) Rhodamine (orange/pink-violet) Rosalic Acid (yellow/brown-red) Rose Bengal (red-yellow) Safanin (red-yellow) SPADNS (varies) Tartrazine (colorless-yellow) Tartrazine Yellow (colorless-yellow) Tashiro's indicator (red/brown-yellow) Tetrabromophenol (yellow-brown) Tetrabromophenolphthalein (yellow-brown) Tetrabromophenolphthalein ethyl ester, potassium salt (yellow-brown) 3,4,5,6-Tetrabromophenolsulfonephthalein (yellow-brown) 3,3,5,5-Tetraiodophenolphthalein (yellow-brown) 3,3,5,5-Tetraiodophenolsulfonephthalein (yellow-brown) Thiazole Yellow G (colorless-yellow) Thorin (red/brown-yellow) Thymol Blue (yellow-blue) Thymol Blue Sodium Salt (red-yellow) Thymolphthalein (colorless-blue) Thymol Violet (yellow-green/violet) Titan Yellow (yellow-red) 4-o-Tolyazo-o-toluidine (orange-yellow) 5,5',7-Trisulfonic acid tripotassium salt (yellow-colorless) 1,3,5-Trinitrobenzene (colorless-orange) 2,4,6-Trinitrobenzene (colorless-orange) 2,4,6 trinitrotoluene (colorless-orange) Tropaeolin (yellow-orange/brown) Universal indicator sticks (varies) Xylene Orange Tetrasodium Salt (red-yellow) Xylenol Blue (red-yellow) Xylenol Orange (red-yellow) Xylenecyanol FF (red-yellow) MIXED INDICATORS: methyl yellow and methylene blue (in alc.) (blue/violet-green) xylene cyanol (in alc.) and methyl orange (in aq.) (violet-green) methyl orange and indigo carmine (in aq.) (violet-green) methyl orange and aniline blue (in aq.) (violet-green) bromocresol green sodium and methyl orange (in aq.) (orange-blue/green) bromocresol green and methyl red (in aq.) (wine/red-green) methyl red and methylene blue (On alc.) red/violet-green) chlorophenol red sodium (in aq.) and aniline blue (in aq.) (green-violet) bromocresol green sodium and chlorophenol red sodium (in aq.) (green-violet) bromocresol purple sodium and bromothymol blue sodium (in aq.) (violet-blue) bromothymol blue sodium and azolitimin (in aq.) (violet-blue) neutral red and methylene blue (in alc.) (violet-blue/green) neutral red and bromothymol blue In alc.) (rose-green) cyanine and phenol red (in alc.) (yellow-violet) bromothymol blue sodium and phenol red sodium (in aq.) (yellow-violet) cresol red sodium and thymol blue sodium (in aq.) (yellow-violet) a-naphtholphthalein and cresol red (in alc.) (pale rose-violet) a-naphtholphthalein and phenolphthalein (in alc.) (pale rose-violet) phenolphthalein and methyl green (in alc.) (green-violet) thymol and phenolphthalein (in alc.) (yellow-violet) phenolphthalein and thymolphthalein (in alc.) (colorless-violet) phenolphthalein and Nile blue (in alc.) (blue-red) thymolphthalein and alizarin yellow (in alc.) (yellow-violet) Nile blue (in aq.) and alizarin yellow (in alc.) (red-brown) SCREENED INDICATORS: Dimethyl yellow and Methylene Blue (pink-yellow green) Methyl orange and Xylene-cyanol (mauve-green) Methyl orange and Aniline green (violet-green) Methyl orange and fluorescein (orange-green) Methyl red and Methylene blue (mauve-green) Chlorophenol red and Aniline Blue (green-violet) Neutral red and Methylene blue (blue violet-green) Phenolphthalein and Methyl green (green-violet) Phenol red and methylene blue (red-blue) Phenolphthalein and methyl green (colorless-green) Phenolphthalein and Nile blue (colorless-blue) Nile blue and alizarin yellow (blue-yellow) UNIVERSAL



## INDICATORS:

Detailed Description Text (18):

There are many examples of universal indicators. They respond over a wide pH range. Examples are: Yamada's universal indicator (pH range 4-10, red-orange-yellow-green-blue-deep blue-violet) van Urk's universal indicator (pH range 2-8, orange/red-red-yellow-green/yellow-green) NATURAL FOOD OR PLANT INDICATORS (derived from Red cabbage and purple cabbage): Red cabbage and purple cabbage (red yellow) Chinese Cabbage Kimchi (red-yellow) Radishes (red-yellow) Red Onions (red-yellow) Strawberrys (red yellow) Blackberries (red-yellow) Rasberries (red-yellow) Cranberries (red-yellow) Grapes (red-yellow) Plums (red-yellow) Cherries (red-yellow) Beets (red-yellow) Carnation flowers (varies) Purple Dahlias (purple-yellow) Purple Hollyhocks (purple -yellow) Red Geraniums (red-yellow) Blue Iris (blue-yellow) Hydrangeas (varies) Roses (varies) Pomergranates (red-yellow) Native/non-poisonous plants (varies) FLUORESCENT INDICATORS: Benzoflavine (yellow-green) 3,6-Dioxypthalimide (blue-green) Eosine YS (yellow color-yellow flock) Erythrosine (yellow color-yellow flock) Esculin (colorless-blue) 4-Ethoxyacridone (green-blue) 3,6-Tetramethyldiaminooxanthone (green-blue) Chromotropic acid (colorless-blue) Fluorescein and derivatives like dichlorofluorescein (colorless-blue) Magdala Red (purple color-purple flock) a-Naphthylamine (colorless-blue) b-Naphthylamine (colorless-violet) Phloxine (colorless-bright yellow) Salicylic acid (colorless-blue) Acridine (green-violet) Dichlorofluorescein (colorless-green) 3,6-Dioxyanthone (colorless-blue/violet) Erythrosine (colorless-yellow/green) b-Methylesculetin (colorless-blue) Neville-Winther acid (colorless-blue) Resorufin (yellow-weak orange) Quininic acid (yellow-blue) Quinine (yellow-violet) Acid R Phosphine (yellow-red) Brilliant Diazol Yellow (colorless-violet) Cleves acid (colorless-green) Coumaric acid (colorless-green) 3,6-Dioxyphthalic dinitrile (blue-green) Magnesium 8-hydroxyquinolate (colorless-golden) b-Methylumbelliferone (colorless-blue) 1-Naphth-4-sulfonic acid (colorless-blue) Orcinaurine ((colorless-green) Patent Phosphine (green-yellow) Thioflavine (yellow color-yellow flock) Umbelliferone (colorless-blue) Acridine Orange (orange-green) Ethoxyphenylnaphthostilbazonium chloride (green color-green flock) G Salt (dull blue-bright blue) Naphthazol derivatives (colorless-yellow/green) a-Naphthionic acid (blue-green) 2-Naphthol-3,6-disulfonic acid (dark blue-light blue) B-Naphthol (colorless-blue flock) a-Naphtholsulfonic acid (dark blue-bright violet) 1,4-Naphthosulfonic acid (dark blue-light blue) Orcinsulfonphthalein (yellow color-yellow flock) Quinine (yellow-black) R-Salt (violet-colorless) Sodium 1-naphthol-2-sulfonate (dark blue-bright violet) Coumarin (deep green-light green) Eosine BN (colorless-yellow) papaverine (permanganate oxidized) (yellow-blue) Schaffers Salt (violet-green/blue) SS-Acid (sodium salt) (violet-yellow) Cotarnine (yellow color-white flock) a-Naphthionic acid (blue-green) b-Naphthionic acid (blue flock-green color) LUMINESCENT INDICATORS: Eosin (colorless-green) Chromotropic acid (colorless-blue) Fluorescein (colorless-green) Dichlorofluorescein (colorless-green) Acridine (green violet) b-Naphthol (blue-violet) Quinine (violet-colorless) IRREVERSIBLE INDICATORS: Amaranth (red-colorless) Bordeaux (faint pink-yellow/green) Brilliant Ponceaux (orange-colorless) Napthol Blue Black (green-faint pink) MISCELLANEOUS INDICATORS: Chemiluminescent indicators such as luminol, lucigenin and lophine (varies) SNARF pH indicators (varies) NERF pH indicators (varies) Dextran indicators (ex. fluoescein tetramethylthrodamine dextran) (varies) Lipophilic indicators (varies) Reactive indicators (varies) Lysosensor indicators (varies) Rhodol derivatives (varies) Radical Scavenger indicators (varies) Coffee extracts (varies) Tea extracts (varies) Herb extracts (varies)

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145-50-6

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alpha-Naphtholbenzein

**RN:** 145-50-6

4-[(4-Hydroxy-1-naphthalenyl)phenylmethylene]-1(4H)-naphthalenone

6948-88-5

p-Naphtholbenzein

**MF:** C27 H18 O2

alpha-Naphtholbenzein indicator solution

C27 H20 O3

Bis-(4-hydroxy-1-naphthyl)phenylmethanol

C27H20O3

alpha-Hydroxy-alpha-(4-hydroxy-1-naphthalenyl)-alpha-phenyl-1-naphthalenemethanol

C27H18O2

4,4'-(alpha-Hydroxybenzylidene)di-1-naphthol

**MW:** 374.43872

p-Naphtolbenzein

392.454

1-naphtholbenzein(ph indicator)

**mp (°C):** 121 - 125

4-(alpha-(4-hydroxy-1-naphthyl)benzylidene)naphthalen-1(4H)-one

a-Naphtholbenzein/4,4'-(a-Hydroxybenzylidene)di-1 -naphthol

P-NAPHTHOLBENZEIN(ALPHA-)

1-Naphtholbenzein

Bis(4-Hydroxy-1-naphthyl)phenylmethanol

Khlorobek

• alpha-Naphtholbenzein

4-[(4-Hydroxy-1-naphthalenyl)phenylmethylene]-1(4H)-naphthalenone

• p-Naphtholbenzein

alpha-Naphtholbenzein indicator solution

Bis-(4-hydroxy-1-naphthyl)phenylmethanol

alpha-Hydroxy-alpha-(4-hydroxy-1-naphthalenyl)-alpha-phenyl-1-naphthalenemethanol

4,4'-(alpha-Hydroxybenzylidene)di-1-naphthol

• p-Naphtholbenzein

• 1-naphtholbenzein(ph indicator)

4-(alpha-(4-hydroxy-1-naphthyl)benzylidene)naphthalen-1(4H)-one

• a-Naphtholbenzein/4,4'-(a-Hydroxybenzylidene)di-1-naphthol

• P-NAPHTHOLBENZEIN(ALPHA-)

• 1-Naphtholbenzein

Bis(4-Hydroxy-1-naphthyl)phenylmethanol

4,4'-bis(dimethylamino)-benzhydrol (Michler's hydrol or BDMB)

Catalog Reference 61131

Supplier AcrosOrganics (Acros Organics)

Iupac name alpha-Naphtholbenzein indicator solution

4-[(4-Hydroxy-1-naphthalenyl)phenylmethylene]-1(4H)-naphthalenone  
p-Naphtholbenzein

Registry number 145-50-6

Supplier catalog information alpha-Naphtholbenzein indicator solution 611315000

500 ML

**Get offer**

**Safety**

Safety 24/25: Avoid contact with skin and eyes.

# United States Patent [19]

Auchincloss

[11] Patent Number: 4,777,018

[45] Date of Patent: Oct. 11, 1988

[54] METHOD OF DISINFECTING PREMISES  
FROM COCCIDIAL OOCYSTS USING  
GENERATED AMMONIA

[76] Inventor: Thomas R. Auchincloss, The Grange,  
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[21] Appl. No.: 14,763

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§ 371 Date: Mar. 23, 1987

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PCT Pub. Date: Dec. 4, 1986

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A01N 25/02; A01N 59/00

[52] U.S. Cl. .... 422/28; 422/29;  
422/32

[58] Field of Search ..... 422/3, 28, 29, 32, 37;  
424/166, 405

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Primary Examiner—Barry S. Richman

Assistant Examiner—Timothy M. McMahon

Attorney, Agent, or Firm—Collard, Roe & Galgano

## [57] ABSTRACT

A method of disinfecting premises from coccidial oocysts in which surface to be disinfected is thoroughly wetted with a first aqueous solution of ammonium salt containing approximately 0.5 to 1.5 molar of ammonium together with non-ionic surfactant and indicator having a color change in the region of pH 8 to pH 10 and the wetted surface is then covered with sufficient of a second aqueous solution of alkali metal hydroxide containing approximately 0.75 to 2.3 molar of hydroxide together with phenolic bactericide to cause the indicator to change color on the treated surface. A preparation for use in such a method comprises a first package containing ammonium salt together with non-ionic surfactant and indicator and second package containing alkali metal hydroxide and phenolic bactericide, the molar amount of hydroxide in the second package being greater than the molar amount of ammonium in the first package.

3 Claims, No Drawings

Indicators

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# METHOD OF DISINFECTING PREMISES FROM COCCIDIAL OOCYSTS USING GENERATED AMMONIA

This invention relates to a method of disinfecting premises from coccidial oocysts. Coccidiosis is a disease affecting livestock particularly poultry especially in a warm humid environment. The disease is caused by certain protozoa referred to generally as coccidia of which various species of *Eimeria* have been identified and shown to be pathogenic to livestock. The disease is spread by oocysts of the coccidia which are resistant to a wide variety of normal disinfectant materials and in many instances can persist in the environment for a very long period of time. For example, oocysts of *Eimeria chandallis*, an important parasite of sheep, are highly resistant to the environment under normal conditions.

In intensive livestock husbandry the premises are generally cleaned and disinfected at the end of each cropping period before re-stocking with young livestock. Unless special precautions are taken, coccidial oocysts left in the environment, particularly on the floor and lower walls of the premises, quickly infect the new livestock to an extent beyond ready control by coccidiostatic medicaments which may be given in the feed.

The most effective chemical agent yet found to control coccidia is ammonia. However the use of aqueous ammonia or ammonia gas is objectionable, not least because of its effects on the operator.

In British patent specification No 1362963 it was proposed to disinfect premises both from coccidia and from bacteria by the use of three components which were added in sequence to water resulting in solubilization of the bactericide and interaction of the chemicals present to produce ammonia gas. In a typical example, sodium hydroxide pellets (728 g, 18.2 moles) were dissolved in water (18.2 liters), ammonium chloride (1110 g, 20.75 moles) was then stirred in and dissolved readily, and finally the bactericide 5,5'-dichloro-2,2'-dihydroxy diphenyl monosulphide (91 ml of 38-42% solution) was added to produce an aqueous disinfectant solution ready for spraying in poultry houses.

However, the procedure described had the drawback that too much gaseous ammonia was released into the atmosphere. This made it necessary for the operator to use a respirator and also depleted the concentration of ammonia at the surfaces where action on the oocysts was required. For good results the premises had to be sealed during and after application of the disinfectant solution.

According to the present invention, there is provided a method of disinfecting premises from coccidial oocysts in which surface to be disinfected is thoroughly wetted with a first aqueous solution of ammonium salt containing approximately 0.5 to 1.5 (preferably 0.8 to 1.0) molar of ammonium together with non-ionic surfactant and indicator having a colour change in the region pH 8 to pH 10 and the wetted surface is then covered with sufficient of a second aqueous solution of alkali metal hydroxide containing approximately 0.75 to 2.3 (preferably 1.2 to 1.5) molar of hydroxide together with phenolic bactericide to cause the indicator to change colour on the treated surfaces.

In the method of the invention, ammonia is liberated only at the surface where it is required, and the amount of ambient gaseous ammonia is very much less than in the earlier method. There is a better disinfecting effect

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against coccidial oocysts, and it is no longer necessary for the operator to use a respirator.

The molar concentration of hydroxide in the second aqueous solution is preferably significantly greater than the molar concentration of ammonium in the first aqueous solution.

The fact that the ammonia is liberated in situ means that the method of the invention can be used effectively in open-sided livestock premises. The reaction liberating ammonia takes place in the infected sites with minimal loss of ammonia and maximum kill of the oocysts. The surfactant in the first aqueous solution ensures penetration of rough surfaces and cracks where oocysts are to be found. The indicator turns colour when the second aqueous solution comes in contact with surfaces wetted with the first aqueous solution and thus guides the operator in his application of the second aqueous solution.

The method of the invention is suitable for poultry, pig, calf and sheep housing, and has resulted in 99.9% reduction in oocysts and 99.9% reduction in bacterial population of infected sites.

The ammonium salt may be one or more of ammonium chloride, ammonium sulphate, and ammonium salts of other inorganic and organic acids.

The alkali metal hydroxide is for economic reasons preferably sodium hydroxide although the hydroxides of potassium and other alkali metals may be used.

The phenolic bactericide may be any such material suitable for killing bacteria in an aqueous alkaline medium. In particular, it may be chosen from the following compounds:

5,5'-dichloro-2,2'-dihydroxy diphenyl monosulphide;  
5,5'-dichloro-2,3'-dihydroxy diphenyl methane;  
p-chloro-m-cresol;  
p-chloro-m-xylene;  
2,4-dichloro-3,5-dimethyl phenol;  
o-phenyl phenol;  
4-chloro-2-phenyl phenol;  
trichlorophenol.

The indicator may be any water-soluble indicator showing a colour change in the range pH 8 to pH 10. Phenolphthalein is particularly suitable because it is colourless at lower pH and becomes intensely red at higher pH thus clearly demonstrating to the operator the areas which have been successfully covered according to the method of this invention. Other indicators which may be used include:

alpha-naphtholphthalein;  
o-cresolphthalein;  
p-naphtholbenzein;  
quinizarin sulphonic acid;  
thymol violet;  
thymolphthalein;  
alizarin yellow GG;  
alizarin yellow.

The surfactant is non-ionic and may for example be one or more of the following:

polyglycol ethers of fatty alcohols;  
fatty acid ethylene oxide condensates;  
polyglycol ethers of alkyl phenols;  
ethylene oxide propylene oxide condensates;  
nonyl phenol ethoxylates;  
fatty alcohol ethoxylates;  
lauryl ether sulphates.

Anionic surfactant may also be present, for example sodium dodecyl sulphonic acid.

4,777,018

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According to the present invention there is also provided for use in the above-described method a preparation comprising two packages, the first package containing ammonium salt together with non-ionic surfactant and indicator and the second package containing an alkali metal hydroxide and phenolic bactericide, the molar amount of hydroxide in the second package being greater than the molar amount of ammonium in the first package preferably by a factor of at least 1.2 but not more than 2.0.

The first package carries instructions for the contents to be dissolved in water the amount of which will be sufficient to give an ammonium concentration of approximately 0.5 to 1.5 molar. This solution is then applied first to the surfaces to be treated.

The second package carries instructions to dissolve the contents in a similar amount of water to give a solution containing approximately 0.75 to 2.3 molar of hydroxide which is then applied as quickly as possible over the surfaces already treated with the solution of the contents of the first package.

The following examples illustrates the invention.

#### EXAMPLE 1

A mixture of the following solid ingredients ammonium chloride (1.4 kg), non-ionic surfactant principally polyglycol ethers of fatty alcohols (63 g) and phenolphthalein (1.2 g) was dissolved in 30 liters of water and applied to 100 square meters of surface to be disinfected.

A solid mixture of sodium hydroxide (1.6 kg) and 5,5'-dichloro-2,2'-dihydroxy diphenyl monosulphide (34 g) was dissolved in 30 liters of cold water and applied as soon as possible onto the same area. This ensured that ammonia was formed in the presence of sufficient water on the surfaces and particularly in the cracks and crevices where coccidial oocysts were to be found.

The amount of ammonia escaping into the atmosphere was relatively very small in comparison with previously known methods of applying ammonia for control of coccidial oocysts. There was no need for the operator to use a respirator.

The above-described procedure was used on a farm in Norfolk England where broiler chickens were raised on earth floors and which had a long history of coccidial infection. Earth plug samples were taken randomly from the floor, six before and six after treatment, and counts were made of oocysts per gramme of soil. The results are given in the following table.

	Before Treatment	After Treatment
1.	100	0
2.	200	0
3.	0	0
4.	300	0
5.	0	0
6.	100	0

The above-described method was also used to disinfect premises on a farm where laying hens were reared on earth floor litter houses. All surfaces were thoroughly cleaned and the entire house and earth floor were power-washed with solutions according to the invention. The disinfected house was then used for the rearing of pullets, and after eighteen weeks the oocysts count was nil. There was also a nil count of ascaridia and capillaria eggs both of which had been present at the level of one hundred eggs per gramme in the un-

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treated floor. The subsequent performance of the birds from the treated house reflected their freedom from internal parasites by better feed utilisation. The disinfection technique of the invention proved to be a valuable aid in lowering the incidence of parasites in the floor-reared pullets on this farm.

#### EXAMPLE 2

The efficacy of the invention was tested against *Eimeria chandallii*, an important parasite of sheep having highly resistant oocysts which cause disease especially when ewes are brought indoors for lambing.

A solid mixture of ammonium chloride (4.78 g), non-ionic surfactant principally polyglycol ethers of fatty alcohols (215 mg) and phenolphthalein (4 mg) was dissolved in 50 ml of water.

A solid mixture of sodium hydroxide (4.90 g) and 5,5'-dichloro-2,2'-dihydroxy diphenyl monosulphide (104 mg) was dissolved in 50 ml of water.

The solutions were combined in the presence of a suspension of oocysts of *Eimeria chandallii* so that the resultant dilution v/v of the original combined solutions was 1/10, 1/100 or 1/500. The diluted solutions containing oocysts were then held at 4° C. or 20° C. and samples were taken after 1 hour, 6 hours and 24 hours to see whether the oocysts had been killed. The oocysts in each sample were washed using several changes of saline. They were then incubated overnight in a solution of sodium bicarbonate (1.4%) to which phenol red had been added, and carbon dioxide was bubbled through until the colour was lost. The oocysts were then washed with saline and resuspended in phosphate-buffered saline at pH 7.6. Glass balls were added and the suspensions were shaken until the shells of the oocysts ruptured releasing sporocytes. A sample was then removed and added to a solution of 0.25% trypsin in 0.5% w/v bile and incubated at 37° C. for up to 45 minutes or until excystment was seen to occur.

The results were as follows ("+"=excystment).

Temperature	Dilution	Time when sample taken		
		1 hour	6 hours	24 hours
4° C.	1/10	-	-	-
4° C.	1/100	+	-	-
4° C.	1/500	+	+	+
20° C.	1/10	-	-	-
20° C.	1/100	+	-	-
20° C.	1/500	+	-	-

These results show that the materials of the invention were effective against *Eimeria chandallii* at a dilution of 1/100 even at 4° C., and at 20° C. all oocysts were killed after one hour's exposure at a dilution of 1/100 or after six hours' exposure at a dilution as high as 1/500.

#### EXAMPLE 3

The efficacy of the invention was tested in preventing the excystment of sporulated avian oocysts.

The sporulated oocysts were obtained from the Parasitology Department of the Central Veterinary Laboratory, Weybridge. The strains of *Eimeria* used in the trial were:

*Eimeria tenella* W264  
*Eimeria necatrix* W71  
*Eimeria brunetti* W63  
*Eimeria acervulina* W102

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Each coccidial suspension was prepared separately in monoculture. Day-old chicks were housed in isolation until 3-4 weeks. During the rearing period faecal samples from the birds were examined twice weekly before oral inoculation with the test strain of oocyst. To prevent cross contamination each species of oocyst was handled in separate isolation facilities by staff having no contact with other birds.

At 5-8 days after inoculation, depending on the species of coccidia, faeces was collected, suspended in water and screened. Finally, the oocysts were harvested by flotation in concentrated sodium chloride solution. The harvested oocysts were then washed and re-suspended in 2% potassium dichromate solution. Sporulation of the oocysts was achieved by incubating the suspensions at 27° C. for 7 days. The final suspensions supplied by the Parasitology Department contained the following proportion of sporulated oocysts:

*Eimeria tenella* 95% sporulated  
*Eimeria necatrix* 85% sporulated  
*Eimeria brunetti* 83% sporulated  
*Eimeria acervulina* 82% sporulated

Before use of each suspension was washed with phosphate buffered saline (PBS) at a pH of 7.6 to remove the potassium dichromate solution. The washed suspensions were standardised to a concentration of 100,000 oocysts per milliliter in PBS.

A solid mixture of ammonium chloride (4.78 g), non-ionic surfactant principally polyglycol ethers of fatty alcohols (215 mg) and phenolphthalein (4 mg) was dissolved in 50ml of water.

A solid mixture of sodium hydroxide (4.90 g) and 5,5'-dichloro-2,2'-dihydroxy diphenyl monosulphide (104 mg) was dissolved in 50 ml of water.

The solutions were combined in the presence of each of a suspension of each avian oocyst so that the resultant dilution v/v of the original combined solutions was neat, 1/10, or 1/100: to test the neat solution of 9 ml of oocyst suspension was re-suspended in a combination of 10 ml of the original combined solutions.

To test the 1/10 solution, 1 ml of original combined solution was added to 9 ml of oocyst suspension.

The efficacy of the 1/100 solution was tested by adding 1 ml of a 1/10 solution of the original combined solutions to 9 ml of oocyst suspension.

The diluted solutions containing oocysts were then held at 4° C. or 20° C. and samples were taken after 1 hour, 6 hours and 24 hours to see whether the oocysts had been killed.

After incubation the diluted solutions containing the oocyst suspensions were centrifuged. Following centrifugation the oocysts were washed several times in PBS. The oocysts were then re-suspended in 1.4% solution of sodium bicarbonate to which phenol red had been added. Carbon dioxide gas was bubbled through until the indicator colour was lost giving a pH of 6.8. After overnight incubation at 37° C. the oocysts were again centrifuged and washed with PBS. They were finally resuspended in PBS. Glass balls were added and the suspension shaken thoroughly. An aliquot of fluid was removed and added to a drop of excysting fluid (0.25% trypsin, with 0.5% bile in PBS). This suspension was incubated at 37° C. for up to 75 minutes or until excystment was seen to occur.

Excystment was deemed not to have occurred if less than 1% of the oocysts excysted. A control suspension of oocysts was handled in parallel with the other proce-

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dures to ensure that the oocyst suspensions were viable. The results were as follows ("+" = excystment):

	Exposure time			Control
	1 h	6 h	24 h	
<i>Eimeria tenella</i> <u>at +4° C. Dilution</u>				
1/1	-	-	-	
1/10	-	-	-	
1/100	+	+	-	+
<u>at +20° C.</u>				
1/1	-	-	-	
1/10	-	-	-	
1/100	+	-	-	+
<i>Eimeria necatrix</i> <u>at +4° C. Dilution</u>				
1/1	-	-	-	
1/10	+	+	-	+
1/100	+	+	+	
<u>at +20° C.</u>				
1/1	-	-	-	
1/10	+	+	-	+
1/100	+	+	+	
<i>Eimeria brunetti</i> <u>at +4° C. Dilution</u>				
1/1	-	-	-	
1/10	+	+	-	+
1/100	+	+	+	
<u>at +20° C.</u>				
1/1	-	-	-	
1/10	+	+	-	+
1/100	+	+	+	
<i>Eimeria acervulina</i> <u>at +4° C. Dilution</u>				
1/1	+	-	-	
1/10	+	+	-	+
1/100	+	+	+	
<u>at +20° C.</u>				
1/1	+	-	-	
1/10	+	+	-	+
1/100	+	+	+	

These results show that the materials of the invention were most effective against *Eimeria tenella*. *Eimeria tenella*, *Eimeria necatrix* and *Eimeria brunetti* could all be inactivated within 1 hour of exposure of the materials.

Although *Eimeria acervulina* showed a greater degree of resistance to inactivation even this strain was inactivated after exposure of between 1 and 6 hours.

There was no significant temperature related effect. The two temperatures were chosen as representative of winter and summer ambient temperatures. Thus the same effects will be found both summer and winter.

I claim:

1. A method of disinfecting a surface from oocysts, which method comprises the steps of:

- (a) combining together an ammonium salt, a surfactant and an indicator having a colour change in the range pH 8 to pH 10 in a first aqueous solution;
- (b) combining together an alkali metal hydroxide and a bactericide in a second aqueous solution;
- (c) applying said first aqueous solution of a surface so as to effect thorough wetting of said surface;
- (d) thereafter applying enough of said second aqueous solution to said surface so that said indicator changes colour indicating the in situ generation of ammonia on said surface; and
- (e) permitting said ammonia to remain in contact with said surface for a time period sufficient to disinfect said surface from oocysts.



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2. A method according to claim 1, wherein said first aqueous solution comprises a 0.5 to 1.5 molar solution of said ammonium salt, and said second aqueous solution comprises a 0.75 to 2.3 molar solution of said alkali metal hydroxide, and the molar concentration of said

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hydroxide is greater than the molar concentration of said ammonium salt.

3. A method according to claim 2, wherein the concentration of the ammonium salt in the first aqueous solution is 0.8 to 1.0 molar and the concentration of hydroxide in the second aqueous solution is 1.2 to 1.5 molar

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DOCUMENT-IDENTIFIER: US 4777018 A

TITLE: Method of disinfecting premises from coccidial oocysts using generated ammonia

Brief Summary Text (25):

p-naphtholbenzein;